AD_____

Award Number: W81XWH-06-1-0424

TITLE: Characterizing Candidate Oncogenes at 8q21 in Breast Cancer

PRINCIPAL INVESTIGATOR: Jessica Kao

CONTRACTING ORGANIZATION: Stanford University
Stanford, Ca 94305

REPORT DATE: March 2007

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
01-03-2007	Annual Summary	1 Mar 2006 – 28 Feb 2007

4. TITLE AND SUBTITLE

Characterizing Candidate Oncogenes at 8q21 in Breast Cancer

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-06-1-0424

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Jessica Kao

Email: jkao@stanford.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Stanford University
Stanford, Ca 94305

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES
Original contains colored plates: ALL DTIC reproductions will be in black and white.

14. ABSTRACT

DNA amplification is a cardinal feature of cancer and plays an important role in tumor progression by altering the gene expression program. These amplified regions are associated with oncogenes of known and unknown identity. By utilizing array comparative genomic hybridization technology to map DNA copy number changes at high resolution, we have identified a recurrent region of high-level amplification at the 8q21 locus that is clinically significant in breast cancer by our preliminary analysis and has yet to be associated with a known oncogene. We have narrowed down this region to two annotated genes with unknown function. In this study we have: (1) created stable cell lines expressing shRNA constructs that will express an siRNA causing inhibition of each candidate oncogene respectively. (2) begun to generate overexpression vectors for each candidate oncogene. (3) raised antibodies to each of the candidate oncogene to further characterize the protein of each candidate oncogenes. Ultimately, this novel oncogene may serve as a new drug target.

15. SUBJECT TERMS
Array CGH

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	11	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

Introduction:
DNA amplification is a cardinal feature of cancer and plays an important role in tumor progression by altering the gene expression program. These amplified regions are associated with oncogenes of known and unknown identity. We aim to identify novel oncogenes within regions of recurrent genomic amplification in breast cancer. By utilizing array comparative genomic hybridization technology to map DNA copy number changes at high resolution, we have identified a recurrent region of high-level amplification at the 8q21 locus that is clinically significant in breast cancer by our preliminary analysis and has yet to be associated with a known oncogene. We narrowed down this region to two annotated genes with unknown function, ZBTB10, and ZNF704, two genes with zinc finger and potential DNA binding domains. These proteins could be transcription factors and potential drug targets.

Body:

Aim 1: Inhibition of candidate oncogene expression and assessment of tumorigenic phenotype.

Two candicate oncogenes, ZBTB10 and ZNF704, were identified from the preliminary microarray data and found to be recurrently amplified in both breast cancer cell lines and tumor samples. The inclusion of both genes within the amplicon was confirmed by real time quantitative PCR in two breast cancer cell lines (SKBR3 and EFM192A). The relevant driver oncogene contributes to the tumorigenic phenotype which can be assessed by measuring cell proliferation, apoptosis, migration, and invasion. We hypothesize that inhibition of the relevant oncogene will result in an altered phenotype whereas inhibition of the irrelevant gene will yield no noticeable change. Therefore, we inhibited both candidate oncogenes respectively by RNAi in breast cancer cell lines and assayed for these phenotypes. A total of 7 siRNA's targeted against ZBTB10 mRNA and 3 siRNA's against ZNF704 were transiently transfected in cell lines that harbored amplification in this region. Successful knockdown of mRNA expression was assessed by real time RT-PCR. The most effective siRNA with the greatest percentage of knockdown

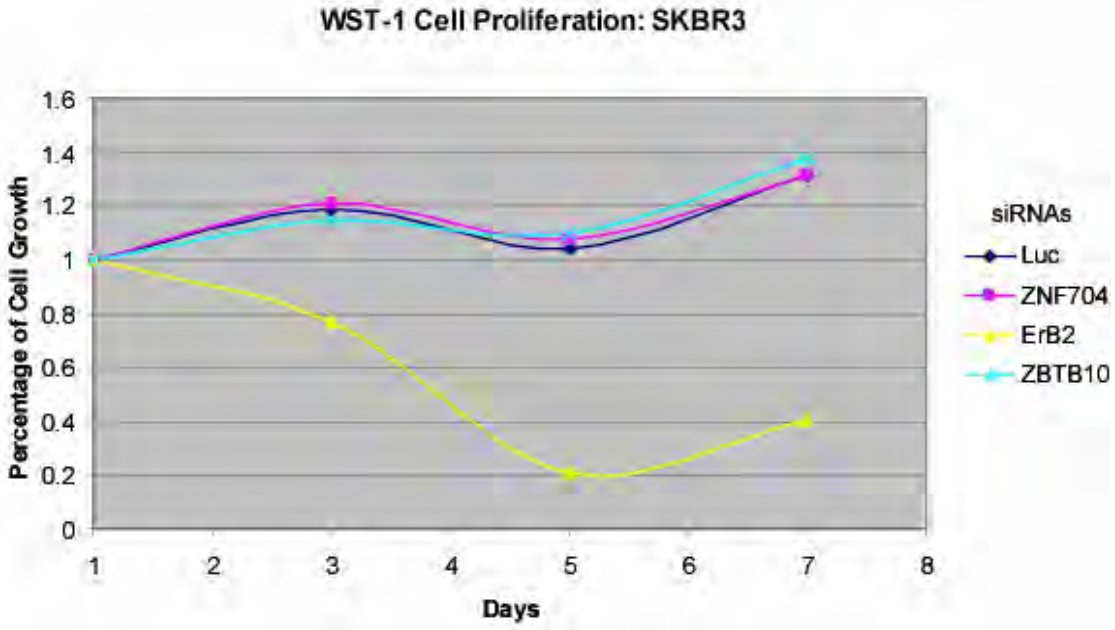

Figure 1. No significant differences in cell proliferation when candidate oncogene expression levels were inhibited. SKBR3 breast cancer cells were transfected with the indicated siRNAs against the candidate oncogenes. Expression of ErbB2 was inhibited as a positive control. siRNA against Luciferase (Luc) was used as a negative control. Cell proliferation was assessed with the WST-1 assay (Roche).

was chosen to conduct further experiments. We were able to achieve 75% knockdown of ZBTB10 gene expression and 50% knockdown of ZNF704 24 hours post transfection. Tumor relevant phenotypes including cell proliferation, apoptosis, and cell migration and invasion were assayed in transiently transfected breast cancer cell lines. However, no significant difference in any of these phenotypes was detected between either candidate oncogene and the negative control siRNA against the irrelevant gene, Luciferase (figure 1).

siRNA transfections in low serum conditions also did not yield any phenotypic changes as previously seen in knockdown experiments with other oncogenes. To ensure, we were achieving knockdown of mRNA levels over the duration of the assay, we assessed mRNA levels for each oncogene on Day1, 3, and 5 post transfection of the siRNA by real time RT-PCR. Although we

achieved significant knockdown of mRNA levels by day 1, ZNF704 expression levels rose on Day 3 and returned to pre-transfection levels by Day 5.

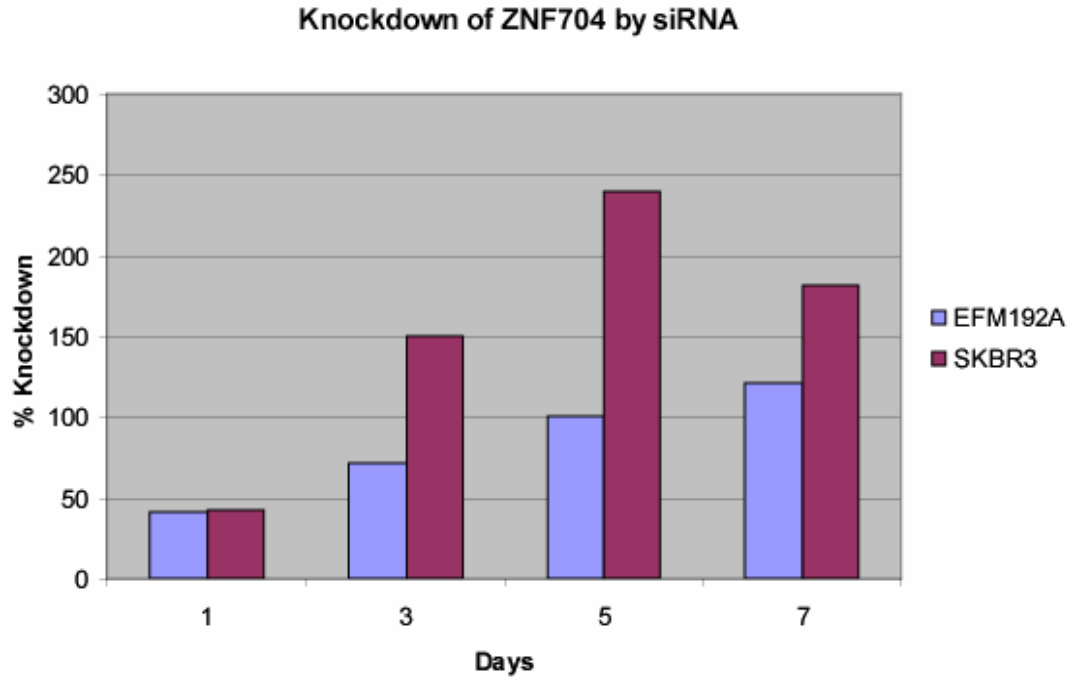

Figure 2. ZNF704 RNA levels rise after transient transfection of siRNA. siRNA targeted against ZNF704 were transefected into two breast cancer cell lines, EFM192A and SKBR3. RNA expressions levels were assessed by real time RT-PCR.

To achieve consistent knockdown, we established stable cell lines that continually suppress expression of the candidate oncogene by stably producing the siRNA. We utilized a construct pSilencer (Ambion) that when transcribed generates a short hairpin (shRNA). . Cells will generate shRNA that will be processed by the cell to produce siRNAs and inhibit expression of the targeted gene over a long period of time. The consistent knock down of gene expression may allow us to detect a phenotypic change. 5 stable cell lines were successfully established for each of the two candidate oncogene. These constructs were transfected into the SKBR3 breast cancer cell line and selected for integration. These 10 stable cell lines will be tested for tumor relevant phenotype such as cell proliferation, cell cycle analysis, apoptosis, and cell migration and invasion

Aim 2: Ectopic overexpression of candidate oncogenes in immortalized breast cancer cell line. cDNAs of each candidate oncogene were obtained by RT-PCR and cloned into mammalian overexpression constructs linked with a v5 epitope tag. Technical difficulties were reached in the expression of the construct when transfected into non-transformed immortialized breast cancer cell line MCF10A. Resequencing and recloning from alternate sources of the gene will be carried out to ensure adequate overexpression of each candidate oncogene.

Aim 3: Characterization of ZNF704 and ZBTB10 protein.
Antibodies were generated against both candidate oncogenes respectively. Antibodies are currently undergoing testing for specificity for both western blot analysis as well as immunohistochemistry. Initial crude lysates did not detect a specific band of the right size during western blotting analysis. Further peptide purification is necessary to yield specificity.

Immunohistochemistry on breast tissue samples as well as paraffin embedded cell lines did not yield specific staining. Further optimization will be needed to overcome these technical difficulties.

Key Research and Training Accomplishments:

- Attended course: Genomics 211
- Attended Introduction to Biostatistics Workshop
- Mastered experimental techniques on both aCGH and gene expression analysis by microarrays.
- Learned to analyze and process microarray data
- Completion of several tutorial courses on the Stanford microarray database.
- Teaching Assistant for Cancer Biology 101

Reportable Outcomes:

Abstract Presentation:

Kao, J. and Pollack J.R. (2007) "The Functional Dissection of Amplicons in Breast Cancer by RNAi." 3rd Annual Asia Pacific Multidisciplinary Metting for Cancer Genomics Research, Hong Kong.

Conclusion:

To determine which candidate oncogene is the 'relevant' driver oncogene in the 8q21 region, rurther characterization will be conducted on stable cell lines with long term inhibition of the candidate oncogene in question. Further optimization of both the cloning of the cDNA of each candidate gene as well as purification of the antibodies will aid in overcoming the technical difficulties encountered.

Appendix:

Curriculum Vitae

Jessica Kao

650-776-1180 • jkao@stanford.edu • 240 Flynn Ave., Mountain View, CA 94043

Education

Stanford University ***2000 – Spring 2007***
PhD in Cancer Biology
Thesis: Identification of novel oncogenes in breast cancer by aCGH and microarray gene expression analysis.
Cumulative 3.7 GPA

Emory University ***1996 - 2000***
B.S. Summa Cum Laude in Biology
B.A. Music
Cumulative 3.7 GPA

Relevant Coursework:
Oral Communication for Graduate Students
Teaching Oral Communication

Research Experience

Stanford University ***2003 - 2007***
PhD candidate, laboratory of Jonathan Pollack
Project: Identification of novel oncogenes in breast cancer by aCGH and microarray gene expression analysis.

- Performed aCGH and gene expression profiling on 50 beast cancer cell lines.
- Performed analysis on parallel DNA/RNA data set.
- Assisted in standardizing new protocol for gene expression on Stanford oligo arrays.
- Consulted with and taught new lab members aCGH protocol.
- Troubleshot standardized, and taught fellow lab members assays for cell proliferation, cell cycle analysis by FACS, siRNA gene knockdown, and apoptosis.
- Extensive experience with validation of array results by qRT-PCR.

Stanford University ***2000 - 2003***
PhD candidate, laboratory of Nic Denko
Project: Characterization of novel hypoxia induced genes

Emory University ***1997 - 2000***
Undergraduate Research, laboratory of Anita Corbett
Project: Cell cycle analysis in *Saccharomyces cerevisiae*

Emory University ***1996 - 1997***
Undergraduate Research, laboratory of Grey Crouse
Project: DNA mismatch repair in *Saccharomyces cerevisiae*

Center for Disease Control – Atlanta, Ga. ***1996 - 1997***
Research Assistant
Project: Diagnostic testing by PCR

Walt Disney World Cancer Research Institute – Orlando, Fl ***1995 - 1996***
Research Assistant

Project: Characterizing CML cell line, K-562

Teaching Experience

Oral Communications Consultant *2005 – Present*

Center for Teaching and Learning, Stanford University

Initiated, designed, and delivered new workshops on oral communication and presentations at the undergraduate and graduate level. Advised and coached undergraduate and graduate students on presentation, interviewing, and speaking skills. Trained other tutors on effective use of powerpoint and teaching oral communication to scientists. Workshops include: Command an audience through posture and body alignment; Common Presentation Pitfalls.

Teaching Assistant

Stanford University

Genomics 109Q: A Technical and Cultural Revolution ***Winter 2007***
Cbio 101 (Cancer Biology) ***Spring 2006***
Bio 42 (Genetics, Biochemistry, and Molecular Biology) & ***Winter 2000***
Led weekly undergraduate discussion sections. Designed, created, and graded assignments.

Emory University

Bio 143 (Genetics) ***Spring 1998***
Led weekly undergraduate discussion sections. Held office hours to aid in students learning.

Dance Instructor

Dance 4 Health non profit ***2006 – Present***

Designed curriculum for after school programs to improve the health and fitness of at risk individuals for obesity and childhood diabetes by integrating nutritional facts with ballroom dance inspired creative movement. Currently developing teacher training curriculum.

Stanford University ***2001 - 2005***

Designed and taught social and competitive ballroom dance classes including: waltz, tango, and salsa.

Leadership Experience

Team Captain, Dance Competition Organizer *2000 - 2006*

Stanford Ballroom Dance Team, Stanford University

Created and organized the implementation of a successful competitive ballroom dance program. Coordinated and organized the planning and execution of the annual ballroom dance competition. Increased revenue by 15% for 3 years in a row. Doubled the attendance from 500 to 1000 spectators the final year. Documented and trained future organizers. Advised other western US collegiate teams in organizational process.

Executive Board Member, Publicity Chair *2005 - 2006*

USA Dance– Northern California Chapter

Assisted in the organization and planning of regional ballroom dance competitions and the 2006 National Ballroom Dancesport Championships. Managed ticket box office, handled customer service issues, and trained volunteers over the 3-day national competition. Increased exposure and attendance of the event through newspaper articles and free advertising. Responsible for media relations.

Freshman Advisor *Fall 2005*

Stanford Farm Mentor, Stanford University

Advised students on both academic and personal issues pertaining to college life.

Board Member ***2001 - 2002***

Graduate Housing Advisory Committee, Stanford University

Liaison between graduate students and the university. Gathered information about student's housing needs through surveys. Presented conclusions to the university committee.

Publications

Kao, J. and Pollack J.R. (2006) "RNA interference-based functional dissection of the 17q12 amplicon in breast cancer reveals contribution of co-amplified genes." Genes, Chromosome, and Cancer; 45(8):761-9.

Abstracts and Presentations

Kao, J. and Pollack J.R. (2007) "The Functional Dissection of Amplicons in Breast Cancer by RNAi." 3rd Annual Asia Pacific Multidisciplinary Metting for Cancer Genomics Research, Hong Kong.

Kao, J. and Pollack J.R. (2005) "Interrogating localized regions of high amplification in breast cancer." Stanford Cancer Biology Conference, Asilomor, Ca.

Kao, J. and Corbett, A. (2000), Mutational analysis of the *Saccharomyces cerevisiae* cyclin-dependent kinase, CDC28. Symposium conducted at The Southeastern Regional Yeast Meeting Birmingham, AL.

Kao, J. and Corbett, A. (1999), Identification of a novel gene implicated in cell cycle regulation in *Saccharomyces cerevisiae*. Symposium conducted at the 39th Annual American Society of Cell Biology Meeting, Washington DC.

Awards and Honors

Department of Defense Breast Cancer Research Pre-Doctoral Training Grant.	***2005***
NIH Cancer Biology Training Grant	***2000-2005***
Howard Hughes Pre-doctoral Fellowship Honorable Mention	***2000***
ODK Leadership Honor Society	***2000***
Mortar Board Senior Honor Society	***2000***
Phi Sigma Biology Honor Society	***2000***
Mu Phi Music Honor Society	***2000***
Howard Hughes Summer Undergraduate Research Fellow	***1998***

Skills

Lab: aCGH on Stanford cDNA and oligo arrays • microarray gene expression analysis • knockdown of gene expression by siRNA • quantitative RT-PCR • FACS analysis • FISH • IHC • immunofluorescence microscopy • tissue culture • western blotting • northern blotting • molecular cloning • standard molecular biology techniques

Computer: Microsoft Excel • Powerpoint • Word • Adobe Photoshop • Illustrator